BIOLOGY

FIFTH EDITION

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(obtained from retroviruses) to make DNA transcripts of this RNA. Each DNA molecule produced carries the coding sequence for a gene but no introns. This DNA is called **complementary DNA**, or **cDNA**, and can be attached to vector DNA for replication inside a cell. Bacteria can express a eukaryotic cDNA gene if the vector provides a bacterial promoter and any other control elements necessary for the gene's transcription and translation.

Molecular biologists can avoid eukaryotic-prokaryotic incompatibility by using eukaryotic cells rather than bacteria as hosts for cloning and/or expressing eukaryotic genes of interest. Yeast cells, single-celled fungi, offer two advantages: They are as easy to grow as bacteria, and they have plasmids, a rarity among eukaryotes. Scientists have even constructed recombinant plasmids that combine yeast and bacterial DNA and can replicate in either type of cell. Scientists have also constructed vectors called **artificial chromosomes** that combine the essentials of a eukaryotic chromosome—an origin for DNA replication, a centromere, and two telomeres—with foreign DNA. These chromosomes behave normally in mitosis, cloning the foreign DNA as the cell divides. An artificial chromosome can carry much more DNA than can a plasmid vector, enabling very long pieces of DNA to be cloned.

Another reason to use eukaryotic host cells for expressing a cloned eukaryotic gene is that many eukaryotic proteins are heavily modified after translation, often by the addition of lipid or carbohydrate groups. Bacterial cells cannot perform any of these processing functions, and if the gene product requiring such processing is from a mammal, even yeast cells will not be able to modify the protein correctly. The use of host cells from an animal or plant cell culture may therefore be necessary.

Like bacteria, many kinds of eukaryotic cells can take up DNA from their surroundings but sometimes not very efficiently. To deal with this problem, scientists have developed a variety of more aggressive methods for introducing recombinant DNA into cells. In **electroporation** they apply a brief electrical pulse to a solution containing cells. The electricity creates temporary holes in the cells' plasma membranes, through which DNA can enter. Alternatively, scientists can inject DNA directly into single eukaryotic cells using microscopically thin needles. And in a technique used primarily for plant cells, they can attach DNA to microscopic particles of metal and fire the particles into cells with a gun (see FIGURE 38.15). Once inside the cell, DNA has a chance to be incorporated into the cell's DNA by natural genetic recombination.

Cloned genes are stored in DNA libraries

Because the gene-cloning procedure in FIGURE 20.3 starts with a mixture of fragments from the entire genome of an organism, it is called a "shotgun" approach—no single gene is targeted for cloning. Thousands of different recombinant plasmids are actually produced in step 2 of FIGURE 20.3, and a clone of each ends up in a (white) colony in step 4. The complete set of thousands of recombinant-plasmid clones, each carrying copies of a particular segment from the initial genome, is referred to as a **genomic library** (FIGURE 20.6, left). A researcher can save such a library and use it as a source of other genes of interest or for genome mapping (as we'll discuss later).

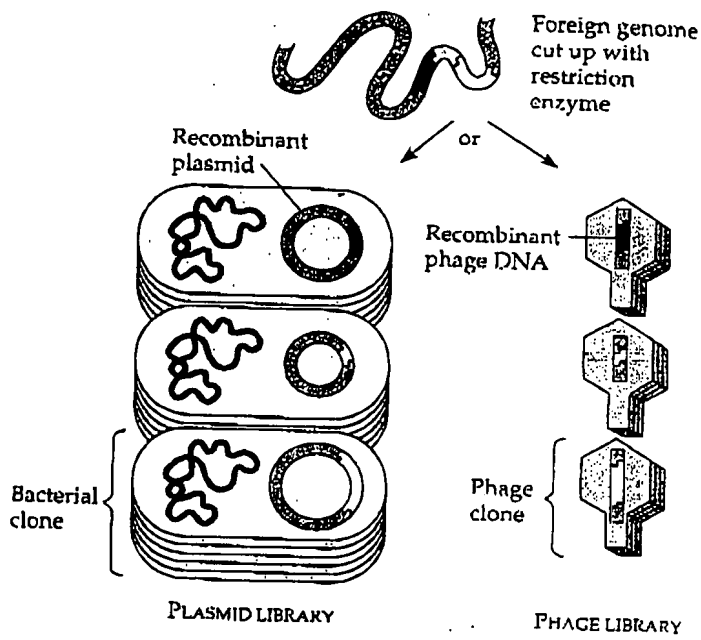


FIGURE 20.6 • Genomic libraries. A genomic library is a collection of a large number of bacterial or phage clones, each containing copies of a DNA segment from a foreign genome. In a complete genomic library, the foreign DNA segments represented cover the entire genome of an organism. This diagram shows parts of two genomic libraries. On the left are three of the thousands of "books" in a plasmid library. Each "book" is a bacterial clone containing one particular variety of foreign genome fragment—red, orange, or yellow here—in its recombinant plasmid. On the right, the same three foreign genome fragments are shown in three "books" of a phage library.

In addition to plasmids, certain bacteriophages are also common cloning vectors for making genomic libraries. Fragments of foreign DNA can be spliced into a phage genome, as into a plasmid, by using a restriction enzyme and ligase. The recombinant phage DNA is then packaged into capsids in vitro and introduced into a bacterial cell through the normal infection process. Once inside the cell, the phage DNA replicates and produces new phage particles, each carrying the foreign DNA. A genomic library made using phage is stored as collections of phage clones (FIGURE 20.6, right). Whatever the cloning vector, restriction enzymes do not respect gene boundaries in cutting up genomic DNA, so some genes in a genomic library may be divided up among two or more clones.

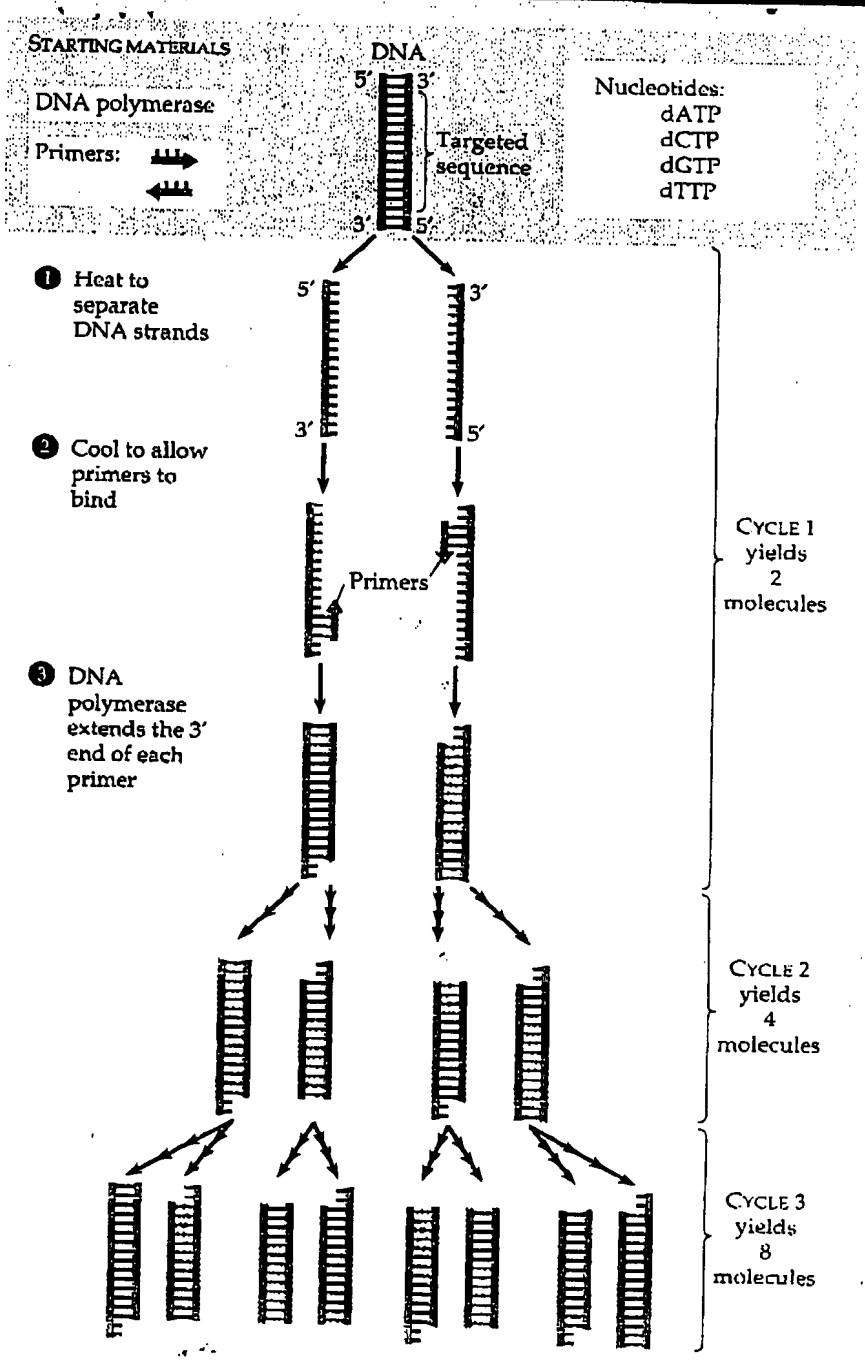
Researchers can make a more limited kind of gene library by using complementary DNA (cDNA). When they isolate mRNA from cells (step 3 in FIGURE 20.5), they actually obtain a mixture of all the mRNA molecules in the cell, transcribed from a number of different genes. Therefore, the cDNA that is

METHODS The Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a method for making many copies of a specific segment of DNA. It is much faster than gene cloning with plasmid or phage DNA and is performed completely in vitro. The starting material for PCR is a solution of double-stranded DNA containing the nucleotide sequence that is "targeted" for copying. The scientist adds a heat-resistant type of DNA polymerase (which catalyzes the reaction), a supply of all four nucleotides (for assembly into new DNA), and primers. The primers are required for the DNA polymerase to initiate DNA synthesis (see Chapter 16). The primers used in PCR are short, synthetic molecules of single-stranded DNA; they are complementary to the ends of the targeted DNA and thus determine the particular segment of DNA to be amplified.

The figure outlines the PCR procedure. (1) The DNA is briefly heated to separate its strands and then (2) cooled to allow the primers to bind by hydrogen bonding to the ends of the target sequence, one primer on each strand. Then (3) the DNA polymerase adds nucleotides to the 3′ ends of the primers, using the longer DNA strands as templates. Within about 5 minutes the targeted DNA sequence—even one hundreds of base pairs long—has been doubled. The solution is then heated again, starting another cycle of strand separation, primer binding, and DNA synthesis. The cycle runs again and again, until the targeted sequence has been duplicated enough times. By about 20 cycles, virtually all the product DNA molecules will consist of the exact targeted sequence (like two of the molecules at the bottom of the figure).

There are practical limits to the number of copies that can be made, often imposed by the accumulation of rare errors in the DNA copies (in vitro versions of mutations). To prepare larger amounts of a DNA segment, PCR can be followed by cloning of the DNA in cells.



made is a library containing a collection of genes. Such a cDNA library represents only part of a cell's genome—only the genes that were transcribed in the starting cells. This is an advantage if a researcher wants to study the genes responsible for specialized functions of a particular kind of cell, such as a brain or liver cell. Also, by making cDNA from cells of the same type at different times in the life of an organism, one can trace changes in patterns of gene expression.

The polymerase chain reaction (PCR) clones DNA entirely in vitro

DNA cloning in cells remains the best method for preparing large quantities of a particular gene or other DNA sequence. However, when the source of DNA is scanty or impure, a method called PCR is quicker and more selective. PCR, the